Exhibit 2

GNE.3230R1C39

PATENT

IN THE STATES PATENT AND TRADEMARK OFFICE

Applicant	:	t al.
Appl. No.	:	57
Filed	:	2002
For	:	TED AND MEMBRANE PTIDES AND NUCLEIC ENCODING THE SAME
Examiner	:	Blanchard
Group Art Unit	:	

DECLARATIC **HRISTOPHER GRIMALDI, UNDER 37 C.F.R. § 1.132**

Commissioner for Pat
P.O. Box 1450
Alexandria, VA 223

Dear Sir:

I, J. Christophe , declare and say as follows:

1. I am a esearch Associate in the Molecular Biology Department of Genentech, Inc., South isco, CA 94080.

2. I joined in January of 1999. From 1999 to 2003, I directed the Cloning Laboratory in the Mo ology Department. During this time I directed or performed numerous molecular b niques including qualitative Polymerase Chain Reaction (PCR) analyses. I am curren ed in, among other projects, the isolation of genes coding for membrane associated hich can be used as targets for antibody therapeutics against cancer. In connectio bove-identified patent application, I personally performed or directed the semi-qu CR analyses in the assay entitled "Tumor Versus Normal Differential Tissue Distribution" which is described in EXAMPLE 18 in the specification that were entify differences in gene expression between tumor tissue and their normal counterpa

3. My sc iculum Vitae, including my list of publications, is attached to and forms part of th (Exhibit A).

4. In diff expression studies, one looks for genes whose expression levels differ significantly un t conditions, for example, in normal versus diseased tissue.

Chromosomal aberr
important markers o
genes and their enc
example, gene ampl
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pattern relative to th
[Singleton *et al.*, Pat
[Grimaldi *et al.*, Bloo
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cancer therapy, for e

h as gene amplification, and chromosomal translocations are
types of cancer and lead to the aberrant expression of specific
eptides, including over-expression and under-expression. For
s a process in which specific regions of a chromosome are
le copies of certain genes that normally exist as a single copy.
ur when a gene is not transcribed into mRNA. In addition,
cur when two different chromosomes break and are rejoined to
in a chimeric chromosome which displays a different expression
romosomes. Amplification of certain genes such as Her2/Neu
27Pt1:165-190], or chromosomal translocations such as t(5;14),
51-2085(1989); Meeker *et al.*, Blood, 76(2):285-289(1990)] give
l advantage relative to normal cells, and might also provide a
nce to chemotherapy or radiotherapy. When the chromosomal
expression of a mRNA and the corresponding gene product (the
foremention cases, the gene product is a promising target for
he therapeutic antibody approach.

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ene expression levels in normal versus diseased tissue has
nostically and therapeutically. For example, those who work in
the vast majority of cases, when a gene is over-expressed, as
ction of mRNA, the gene product or polypeptide will also be
one identifies increased mRNA expression without associated
is same principle applies to gene under-expression. When a
product is also likely to be under-expressed. Stated in another
ave differing mRNA concentrations for a specific gene are
different concentration of protein for that gene. Techniques
Northern Blotting, Differential Display, *in situ* hybridization,
ore recently Microarray technology all rely on the dogma that a
similar change in protein. If this dogma did not hold true then
ble value and not be so widely used. The use of mRNA
lentified a seemingly endless number of genes which are
tissues and these genes have subsequently been shown to have
their protein levels. Thus, the detection of increased mRNA
in increased polypeptide expression, and the detection of
xpected to result in decreased polypeptide expression. The
polypeptide expression can be used for cancer diagnosis and

6. Ho
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and treatment. F
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he rare case where the protein expression does not correlate
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er- or under-expression of a gene product does not correlate
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sion and protein expression enables more accurate tumor
rmination of suitable therapy. In addition, absence of over- or

under-expression of t ..ict in the presence of a particular over- or under-expression of mRNA is crucial in.' ..e practicing clinician. For example, if a gene is over-expressed but the correspondi: e. ..t is not significantly over-expressed, the clinician accordingly will decide not to t: ..th agents that target that gene product.

7. I her. ..t all statements made herein of my own knowledge are true and that all statements m: ..nation or belief are believed to be true, and further that these statements were m.. ..owledge that willful false statements and the like so made are punishable by fine .. ..., or both, under Section 1001 of Title 18 of the United States Code and that suc.. ..ents may jeopardize the validity of the application or any patent issued there..

By: _____
J. Christo...

Date: 8/10/2004

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Exhibit 1

GNE.3230R1C39 PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Eaton, et al.
Appl. No.	:	10/063,557
Filed	:	May 2, 2002
For	:	SECRETED AND TRANSMEMBRANE POLYPEPTIDES AND NUCLEIC ACIDS ENCODING THE SAME
Examiner	:	David J. Blanchard
Group Art Unit	:	1642

DECLARATION OF J. CHRISTOPHER GRIMALDI, UNDER 37 CFR §1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

I, J. Christopher Grimaldi, declare and state as follows:

1. I am a Senior Research Associate in the Molecular Biology Department of Genentech, Inc., South San Francisco, CA 94080.

2. My scientific Curriculum Vitae, including my list of publications, is attached to and forms part of this Declaration (Exhibit A).

3. I joined Genentech in January of 1999. From 1999 to 2003, I directed the Cloning Laboratory in the Molecular Biology Department. During this time I directed or performed numerous molecular biology techniques including semi-quantitative Polymerase Chain Reaction (PCR) analyses. I am currently involved, among other projects, in the isolation of genes coding for membrane associated proteins which can be used as targets for antibody therapeutics against cancer. In connection with the above-identified patent application, I personally performed or directed the semi-quantitative PCR gene expression analyses in the assay entitled "Tumor Versus Normal Differential Tissue Expression Distribution," which is described in EXAMPLE 18 in the specification. These studies were used to identify differences in gene expression between tumor tissue and their normal counterparts.

4. EXAMPLE 18 reports the results of the PCR analyses conducted as part of the investigating of several newly discovered DNA sequences. This process included developing

primers and analyzing expression of the DNA sequences of interest in normal and tumor tissues. The analyses were designed to determine whether a difference exists between gene expression in normal tissues as compared to tumor in the same tissue type.

5. The DNA libraries used in the gene expression studies were made from pooled samples of normal and of tumor tissues. Data from pooled samples is more likely to be accurate than data obtained from a sample from a single individual. That is, the detection of variations in gene expression is likely to represent a more generally relevant condition when pooled samples from normal tissues are compared with pooled samples from tumors in the same tissue type.

6. In differential gene expression studies, one looks for genes whose expression levels differ significantly under different conditions, for example, in normal versus diseased tissue. Thus, I conducted a semi-quantitative analysis of the expression of the DNA sequences of interest in normal versus tumor tissues. Expression levels were graded according to a scale of +, -, and +/- to indicate the amount of the specific signal detected. Using the widely accepted technique of PCR, it was determined whether the polynucleotides tested were more highly expressed, less expressed, or whether expression remained the same in tumor tissue as compared to its normal counterpart. Because this technique relies on the visual detection of ethidium bromide staining of PCR products on agarose gels, it is reasonable to assume that any detectable differences seen between two samples will represent at least a two fold difference in cDNA.

7. The results of the gene expression studies indicate that the genes of interest can be used to differentiate tumor from normal. The precise levels of gene expression are irrelevant; what matters is that there is a relative difference in expression between normal tissue and tumor tissue. The precise type of tumor is also irrelevant; again, the assay was designed to indicate whether a difference exists between normal tissue and tumor tissue of the same type. If a difference is detected, this indicates that the gene and its corresponding polypeptide and antibodies against the polypeptide are useful for diagnostic purposes, to screen samples to differentiate between normal and tumor. Additional studies can then be conducted if further information is desired.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information or belief are believed to be true, and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful statements may jeopardize the validity of the application or any patent issued thereon.

By: _____ Date: 8/10/2004

J. Christopher Grimaldi

S:\DOCS\AOK\AOK-5591.DOC/081004

J. Christopher Grimaldi

1434-36th Ave.
San Francisco, CA 94122
(415) 681-1639 (Home)

EDUCATION

University of California, Berkeley
Bachelor of Arts in Molecular Biology, 1984

EMPLOYMENT EXPERIENCE

SRA — Genentech Inc., South San Francisco; 1/99 to present

Previously, was responsible to direct and manage the Cloning Lab. Currently focused on isolating cancer specific genes for the Tumor Antigen (TAP), and Secreted Tumor Protein (STOP) projects for the Oncology Department as well as Immunologically relevant genes for the Immunology Department. Directed a lab of 6 scientists focused on a company-wide team effort to identify and isolate secreted proteins for potential therapeutic use (SPDI). For the SPDI project my duties were, among other things, the critically important coordination of the cloning of thousands of putative genes, by developing a smooth process of communication between the Bioinformatics, Cloning, Sequencing, and Legal teams. Collaborated with several groups to discover novel genes through the Curagen project, a unique differential display methodology. Interacted extensively with the Legal team providing essential data needed for filing patents on novel genes discovered through the SPDI, TAP and Curagen projects. My group has developed, implemented and patented high throughput cloning methodologies that have proven to be essential for the isolation of hundreds of novel genes for the SPDI, TAP and Curagen projects as well as dozens of other smaller projects.

Scientist — DNAX Research Institute, Palo Alto; 9/91 to 1/99

Involved in multiple projects aimed at understanding novel genes discovered through bioinformatics studies and functional assays. Developed and patented a method for the specific depletion of eosinophils in vivo using monoclonal antibodies. Developed and implemented essential technical methodologies and provided strategic direction in the areas of expression, cloning, protein purification, general molecular biology, and monoclonal antibody production. Trained and supervised numerous technical staff.

Facilities Manager — Corixa, Redwood City; 5/89 - 7/91.

Directed plant-related activities, which included expansion planning, maintenance, safety, purchasing, inventory control, shipping and receiving, and laboratory management. Designed and implemented the safety program. Also served as liaison to regulatory agencies at the local, state and federal level. Was in charge of property leases, leasehold improvements, etc. Negotiated vendor contracts and directed the purchasing department. Trained and supervised personnel to carry out the above-mentioned duties.

SRA University of California, San Francisco
Cancer Research Institute; 2/87-4/89.

Was responsible for numerous cloning projects including: studies of somatic hypermutation, studies of AIDS-associated lymphomas, and cloning of t(5;14), t(11;14), and t(8;14) translocations. Focused on the activation of hemopoietic growth factors involved in the t(5;14) translocation in leukemia patients..

Research Technician Berlex Biosciences, South San Francisco; 7/85-2/87.

Worked on a subunit porcine vaccine directed against Mycoplasma hyopneumoniae. Was responsible for generating genomic libraries, screening with degenerate oligonucleotides, and characterizing and expressing clones in E. coli. Also constructed a general purpose expression vector for use by other scientific teams.

PUBLICATIONS

1. Hilary F. Clark, et al. "The Secreted Protein Discovery Initiative (SPDI), a Large-scale Effort to Identify Novel Human Secreted and Transmembrane Proteins: a bioinformatics assessment." Genome Res. Vol 13(10), 2265-2270, 2003

2. Sean H. Adams, Clarissa Chui Sarah L. Schilbach, Xing Xian Yu, Audrey D. Goddard, J. Christopher Grimaldi, James Lee, Patrick Dowd, David A. Lewin, & Steven Colman' 'BFIT, a Unique Acyl-CoA Thioesterase Induced in Thermogenic Brown Adiopose Tissue: Cloning, organization of the humanb gene and assessment of a potential link to obesity" Biochemical Journal, Vol 360, 135-142, 2001

3. Szeto W, Jiang W, Tice DA, Rubinfeld B, Hollingshead PG, Fong SE, Dugger DL, Pham T, Yansura D, Wong TA, Grimaldi JC, Corpuz RT, Singh JS, Frantz GD, , Devaux B, Crowley CW, Schwall RH, Eberhard DA, Rastelli L, Polakis P, and Pennica D. "Overexpression of the Retenoic Acid-Responsive Gene Stra6 in Human Cancers and its Synergistic Activation by Wnt-1 and Retinoic Acid." Cancer Research Vol. 61(10), 4197-4205, 2001

4. Jeanne Kahn, Fuad Mehraban, Gladdys Ingle, Xiaohua Xin, Juliet E. Bryant, Gordon Vehar, Jill Schoenfeld, J. Christopher Grimaldi (incorrectly named as "Grimaldi, CJ"), Franklin Peale, Aparna Draksharapu, David A. Lewin, and Mary E. Gerritsen. "Gene Expression Profiling in an in Vitro Model of Angiogenesis." American Journal of Pathology Vol 156(6), 1887-1900, 2000.

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9. M. J. Guimaraes, J. F. Bazan, A. Zolotnik, M. V. Wiles, J. C. Grimaldi, F. Lee, T. McClanahan. "A new approach to the study of haematopoietic development in the yolk sac and embryoid body." Development, Vol. 121(10), 3335-3346, 1995

10. J. Christopher Grimaldi, Sriram Balasubramanian, J. Fernando Bazan, Armen Shanafelt, Gerard Zurawski and Maureen Howard. "CD38-mediated protein ribosylation." Journal of Immunology,Vol. 155(2), 811-817, 1995

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14. Nobuyuki Harada, Leopoldo Santos-Argumedo, Ray Chang, J. Christopher Grimaldi, Frances Lund, Camilynn I. Brannan, Neal G. Copeland, Nancy A. Jenkins, Andrew Heath, R. M. E. Parkhouse and Maureen Howard. "Expression Cloning of a cDNA Encoding a Novel Murine B Cell Activation Marker: Homology to Human CD38." The Journal of Immunology, Vol. 151, 3111-3118, 1993

15. David J. Rawlings, Douglas C. Saffran, Satoshi Tsukada, David A. Largaespada, J. Christopher Grimaldi, Lucie Cohen Randolph N. Mohr, J. Fernando Bazan, Maureen Howard, Neal G. Copeland, Nancy A. Jenkins, Owen Witte. "Mutation of Unique Region of Bruton's Tyrosine Kinase in Immunodeficient XID Mice." Science, Vol. 261, 358-360, 1993

16. J. Christopher Grimaldi, Raul Torres, Christine A. Kozak, Ray Chang, Edward Clark, Maureen Howard, and Debra A. Cockayne. "Genomic Structure and Chromosomal Mapping of the Murine CD40 Gene." The Journal of Immunology, Vol 149, 3921-3926, 1992

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Division into Peripheral Monoclonal, Polyclonal and Central Nervous System Lymphoma." AIDS, Vol. 5, 669-674, 1991

18. Ann Grimaldi and Chris Grimaldi. "Small-Scale Lambda DNA Prep." Contribution to Current Protocols in Molecular Biology, Supplement 5, Winter 1989

19. J. Christopher Grimaldi, Timothy C. Meeker. "The t(5;14) Chromosomal Translocation in a Case of Acute Lymphocytic Leukemia Joins the Interleukin-3 Gene to the Immunoglobulin Heavy Chain Gene." Blood, Vol. 73, 2081-2085, 1989

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21 Timothy C. Meeker, J. Christopher Grimaldi, Robert O'Rourke, et al. "Lack of Detectable Somatic Hypermutation in the V Region of the Ig H Chain Gene of a Human Chronic B Lymphocytic Leukemia." The Journal of Immunology, Vol. 141, 3994-3998, 1988

MANUSCRIPTS IN PREPARATION

1. Sriram Balasubramanian, J. Christopher Grimaldi, J. Fernando Bazan, Gerard Zurawski and Maureen Howard. "Structural and functional characterization of CD38: Identification of active site residues"

PATENTS

1. "Methods for Eosinophil Depletion with Antibody to CCR3 Receptor" (US 6,207,155 B1).

2. "Amplification Based Cloning Method." (US 6,607,899)

3. Ashkenazi et al., "Secreted and Transmembrane Polypeptides and Nucleic Acids Encoding the Same." (this patent covers several hundred genes)

4. "IL-17 Homologous Polypeptides and Therapeutic Uses Thereof"

5. "Method of Diagnosing and Treating Cartilaginous Disorders."

MEMBERSHIPS AND ACTIVITIES

Editor	Frontiers in Bioscience
Member	DNAX Safety Committee 1991-1999 Biological Safety Affairs Forum (BSAF) 1990-1991 Environmental Law Foundation (ELF) 1990-1991

The t(5;14) Chromosomal Translocation in a Case of Acute Lymphocytic Leukemia Joins the Interleukin-3 Gene to the Immunoglobulin Heavy Chain Gene

By J. Christopher Grimaldi and Timothy C. Meeker

Chromosomal translocations have proven to be important markers of the genetic abnormalities central to the pathogenesis of cancer. By cloning chromosomal breakpoints one can identify activated proto-oncogenes. We have studied a case of B-lineage acute lymphocytic leukemia (ALL) that was associated with peripheral blood eosinophilia. The chromosomal translocation t(6;14) (q31;q32) from this sample was cloned and studied at the molecular level. This translocation joined the immunoglobulin heavy chain joining (Jh) region to the promotor region of the interleukin-3 (IL-3) gene in opposite transcriptional orientations. The data suggest that activation of the IL-3 gene by the enhancer of the immunoglobulin heavy chain gene may play a central role in the pathogenesis of this leukemia and the associated eosinophilia.

KARYOTYPIC STUDIES of leukemia and lymphoma have identified frequent nonrandom chromosomal translocations. Some of these translocations juxtapose the immunoglobulin heavy chain (IgH) gene with important protooncogenes, such as c-*myc* and *bcl*-2.[1,2] In this way, the IgH gene can activate proto-oncogenes, resulting in disordered gene expression and a step in the development of cancer. The investigation of additional nonrandom translocations into the IgH locus allows us to identify new genes promoting the generation of leukemia and lymphoma.

A distinct subtype of acute lymphocytic leukemia (ALL) has been characterized by B-lineage phenotype, associated eosinophilia in the peripheral blood, and a t(5;14)(q31;q32) chromosomal translocation.[3,4] This syndrome probably occurs in <1% of all patients with ALL. We hypothesized that the cloning of the translocation characteristic of this leukemia might allow the identification of an important gene on chromosome 5 that plays a role in the evolution of this disease. In this report we demonstrate that the interleukin-3 gene (IL-3) and the IgH gene are joined by this translocation.

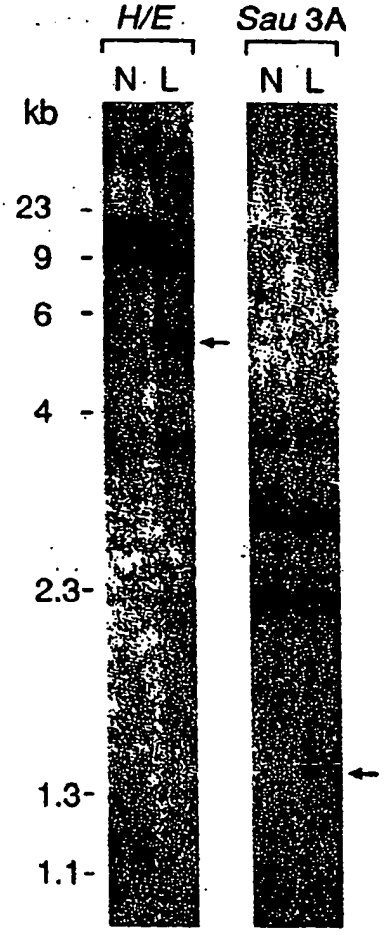

Fig 1. DNA blots of the leukemia sample. The restriction fragment pattern of normal human DNA (N) and the leukemia sample (L) were compared using a human Jh probe. Rearranged bands are indicated by arrows. Sample L exhibits a single rearranged band with both *Hind* III/*Eco*RI and *Sau*3A restriction digests. The rearranged bands are less intense than the other bands because the majority of cells in the sample represent normal bone marrow elements.

MATERIALS AND METHODS

Sample and DNA blots. A bone marrow aspirate from a representative patient with ALL (L1 morphology by French-American-British [FAB] criteria), peripheral eosinophilia (up to 20,000 per microliter with a normal value of <350 per microliter) and a t(5;14)(q31;q32) translocation was studied. Using published methods, genomic DNA was isolated and DNA blots were made.[5] Briefly, 10 μg of high molecular weight (mol wt) DNA were digested using an appropriate restriction enzyme and electrophoresed on a 0.8% agarose gel. The gel was stained with ethidium bromide, photographed, denatured, neutralized, and transferred to Hybond (Amersham, Arlington Heights, IL). After treatment of the filter with ultraviolet light, hybridization was performed. The filter was washed to a final stringency of 0.2% saturated sodium citrate (SSC) and 0.1% sodium lauryl sulfate (SDS) and exposed to film. The human Jh probe has been previously reported.[6]

Genomic library. The genomic library was made using pub-

From the Division of Hematology/Oncology, Department of Medicine, University of California, San Francisco.

Submitted February 22, 1989; accepted March 8, 1989.

Supported by NIH Grant No. CA01102.

Address reprint requests to Timothy C. Meeker, MD, UCSF/VAMC 111H, 4150 Clement St, San Francisco, CA 94121.

Dr Grimaldi's current address is Biostan Inc, 440 Chesapeake Dr, Seaport Centre, Redwood City, CA 94063.

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0006-4971/89/7308-0031$3.00/0

lished methods.[5] Approximately 100 μg of high mol wt genomic DNA were partially digested with the *Sau*3A restriction enzyme. Fragments from 9 to 23 kilobases (kb) in size were isolated on a sucrose gradient and ligated into phage EMBL3A (Strategene, San Diego). Recombinant phage were packaged, plated, and screened as previously reported.[5]

DNA sequencing. Fragments for sequencing were cloned into M13 vectors and sequenced by the chain termination method using Sequenase (United States Biochemical, Cleveland).[7] All sequence data were derived from both strands.

RESULTS

We studied a bone marrow sample from a patient with ALL and associated peripheral eosinophilia. Karyotypic analysis showed the characteristic t(5;14)(q31;q32) translocation. These features define a distinctive subtype of ALL.[3,4] The leukemic cells were analyzed for cell surface phenotype by immunofluorecence. They were positive for B1 (CD20), B4 (CD19), cALLA (CD10), HLA-DR, and terminal deoxynucleotidyl transferase (Tdt), but negative for surface immunoglobulin. This phenotypic profile describes an immature cell from the B-lymphocytic lineage.[8]

The leukemia DNA was analyzed by Southern blotting for rearrangements of the IgH gene. Using a human immunoglobulin Jh probe, a single rearranged band was detected by *Eco*RI, *Hind*III, *Sst*I, *Sau*3A, and *Eco*RI plus *Hind*III restriction digests, suggesting rearrangement of one allele (Fig 1). The immunoglobulin Jh region from the other allele was presumably either deleted or in the germline configuration.

We hypothesized that the t(5;14)(q31;q32) juxtaposed a growth-promoting gene on chromosome 5 with the immunoglobulin Jh region on chromosome 14. Therefore, a genomic library was made from the leukemic sample and screened with a Jh probe. Fifteen distinct positive clones were isolated and screened for the presence of the rearranged *Sau*3A fragment that was detected by DNA blotting. By this analysis, five clones appeared to represent the rearranged allele identified by DNA blots. One of these clones (clone no. 4) was chosen for further study and a detailed restriction map was generated. The *Eco*RI, *Hind*III/*Eco*RI, and *Sst*I fragments from clone no. 4 that hybridized to the human Jh probe were also identical in size to the rearranged fragments from the leukemia sample, confirming that clone no. 4 represented the rearranged leukemic allele.

Phage clone no. 4 contained 3.7 kb of unknown origin joined to the IgH gene in the region of Jh4 (Fig 2). The IgH gene from Jh4 to the Cmu region appeared to be in germline configuration. Previously, the gene encoding hematopoietic growth factor IL-3 had been mapped to chromosome 5q31 so it was suspected that clone no. 4 might contain part of this gene.[9-12] When the restriction map of human IL-3 and clone no. 4 were compared, they were identical for more than 3 kb (Fig 2).

We confirmed the juxtaposition of the IL-3 gene and the IgH gene by nucleic acid sequencing of the subcloned *Bst*EII/*Hpa*I fragment (Fig 2). The sequence of this fragment showed no disruption of the protein coding region or the messenger RNA of the IL-3 gene. The break in the IL-3 gene occurred in the promotor region, 452 base pairs (bp) upstream of the transcriptional start site (position 64, Fig

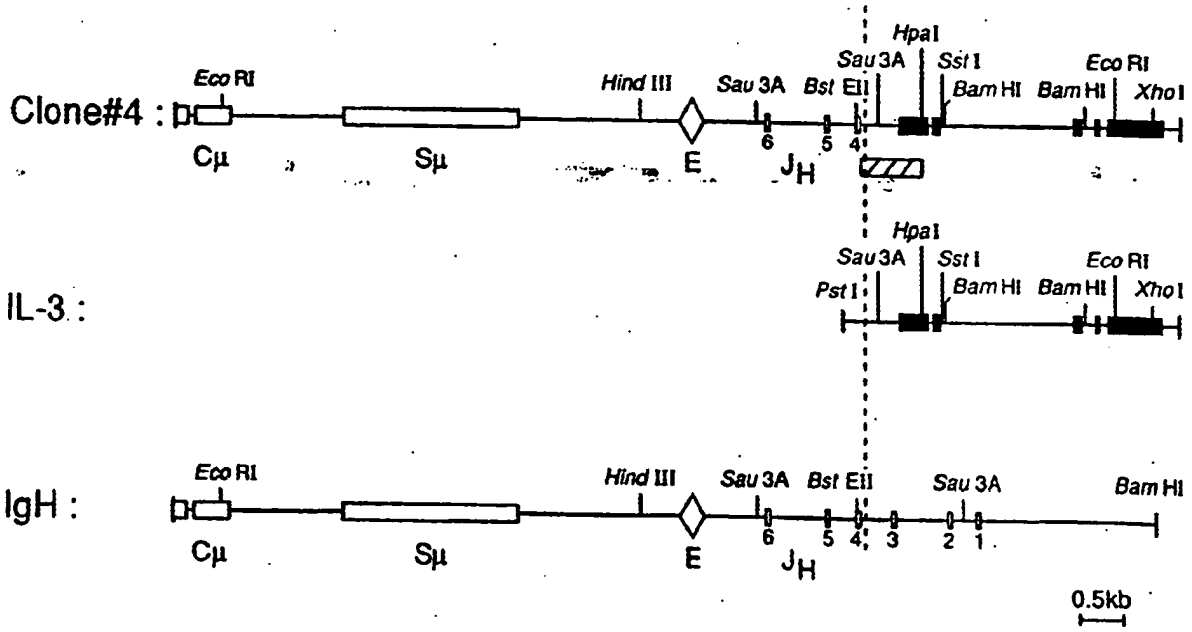

Fig 2. Breakpoint region: t(5;14)(q31;q32). Comparative mapping of phage clone no. 4, the germline IgJh region, and the germline IL-3 gene.[10,12] The map of clone no. 4 is identical to that of IgH until it diverges in the region of Jh4 (at the dashed line), after which it is identical to the map of IL-3. The two genes are positioned in a head-to-head orientation. The Ig μ chain constant region (Cμ), switch region (Sμ), enhancer (E), and Jh segments are indicated (open symbols). The five exons (dark boxes) and four introns of the IL-3 gene are shown. The hatched box indicates the sequenced region.

3A). The break in the IgH gene occurred 2 bp upstream of the Jh4 region. Between the two breaks, 25 bp of uncertain origin (putative N sequence) were inserted.[13,14] No sequences homologous to the immunoglobulin heptamer and nonamer could be identified in the IL-3 sequence (Fig 3B). Therefore, nucleic acid sequencing confirmed the juxtaposition of the IL-3 gene and the IgH gene. The sequence data clearly showed that the genes were positioned in opposite transcriptional orientations (head-to-head).

Available data also allowed us to determine the normal positions of the IL-3 gene and the GM-CSF gene in relation to the centromere of chromosome 5 (Fig 4). The IgH gene is known to be positioned with the variable regions toward the telomere on chromosome 14q.[2,15] It has also been shown that GM-CSF maps within 9 kb of IL-3 in the same transcriptional orientation.[16] Using this information and assuming a simple translocation event in our sample, we can conclude that the IL-3 gene is normally more centromeric, and the GM-CSF gene more telomeric on chromosome 5q (Fig 4). Furthermore, both are transcribed with their 5′ ends toward the centromere.

DISCUSSION

In this report we have cloned a unique chromosomal translocation that appears to be a consistent feature of a rare, yet distinct, clinical form of acute leukemia. This translocation joined the promotor of the IL-3 gene to the IgH gene. Except for the altered promotor, the IL-3 gene appeared

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                                                                  +
5'GGTGACCAGGGTTCCCTGGCCCCAGTAGTCAAAGTAGTAGAGGTAATTCATCATAGCTGCGGATTAGCAGCGTGACCGGC   80
3'CCACTGGTCCCAAGGGACCGGGGTCATCAGTTTCATCATCTCCATTAAGTAGTATCGACGCCTAATCGTCGCACTGGCCG

5'TACCAGACAAACTCTCATCTGTTCCAGTGGCCTCCTGGCCACCCACCAGGACCAAGCAGGGCGGGCAGCAGAGGGCCAGG  160
3'ATGGTCTGTTTGAGAGTAGACAAGGTCACCGGAGGACCGGTGGGTGGTCCTGGTTCGTCCCGCCCGTCGTCTCCCGGTCC

                  *********
5'GTAGTCCAGGTGATGGCAGATGAGATCCCACTGGGCAGGAGGCCTCAGTGAGCTGAGTCAGGCTTCCCCTTCCTGCCACA  240
3'CATCAGGTCCACTACCGTCTACTCTAGGGTGACCCGTCCTCCGGAGTCACTCGACTCAGTCCGAAGGGGAAGGACGGTGT

5'GGGGTCCTCTCACCTGCTGCCATGCTTCCCATCTCTCATCCTCCTTGACAAGATGAAGTGATACCGTTTAAGTAATCTTT  320
3'CCCCAGGAGAGTGGACGACGGTACGAAGGGTAGAGAGTAGGAGGAACTGTTCTACTTCACTATGGCAAATTCATTAGAAA

                                                        *********
5'TTTCTTGTTTCACTGATCTTGAGTACTAGAAAGTCATGGATGAATAATTACGTCTGTGGTTTTCTATGGAGGTTCCATGT  400
3'AAAGAACAAAGTGACTAGAACTCATGATCTTTCAGTACCTACTTATTAATGCAGACACCAAAAGATACCTCCAAGGTACA

5'CAGATAAAGATCCTTCCGACGCCTGCCCCACACCACCACCTCCCCCCGCCTTGCCCGGGGTTGTGGGCACCTTGCTGCTG  480
3'GTCTATTTCTAGGAAGGCTGCGGACGGGGTGTGGTGGTGGAGGGGGGCGGAACGGGCCCCAACACCCGTGGAACGACGAC

5'CACATATAAGGCGGGAGGTTGTTGCCAACTCTTCAGAGCCCCACGAAGGACCAGAACAAGACAGAGTGCCTCCTGCCGAT  560
3'GTGTATATTCCGCCCTCCAACAACGGTTGAGAAGTCTCGGGGTGCTTCCTGGTCTTGTTCTGTCTCACGGAGGACGGCTA

5'CCAAACATGAGCCGCCTGCCCGTCCTGCTCCTGCTCCAACTCCTGGTCCGCCCCGGACTCCAAGCTCCCATGACCCAGAC  640
3'GGTTTGTACTCGGCGGACGGGCAGGACGAGGACGAGGTTGAGGACCAGGCGGGGCCTGAGGTTCGAGGGTACTGGGTCTG

5'AACGTCCTTGAAGACAAGCTGGGTTAAC 3'
3'TTGCAGGAACTTCTGTTCGACCCAATTG 5'  668
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B

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IgJh4   5'TGGCCCCAGTAGTCAAAGTAGTCACATTGTGGGAGGCCCCATTAAGGGGTGCACAAAAACCTGACTCTC
        3'ACCGGGGTCATCAGTTTCATCAGTGTAACACCCTCCGGGGTAATTCCCCACGTGTTTTTGGACTGAGAG
           ++++++++++++++++++++++

C1.#4   5'TGGCCCCAGTAGTCAAAGTAGTAGAGGTAATTCATCATAGCTGCGGATTAGCAGCGTGACCGGCTACCA
        3'ACCGGGGTCATCAGTTTCATCATCTCCATTAAGTAGTATCGACGCCTAATCGTCGCACTGGCCGATGGT
                                                  ++++++++++++++++++++++

Il-3    5'GGCACCAAGAGATGTGCTTCTCAGAGCCTGAGGCTGAACGTGGATGTTTAGCAGCGTGACCGGCTACCA
        3'CCGTGGTTCTCTACACGAAGAGTCTCGGACTCCGACTTGCACCTACAAATCGTCGCACTGGCCGATGGT
```

Fig 3. Sequence of t(5;14)(q31;q32) breakpoint region. (A) Nucleotide sequence of the *Bst*EII/*Hpa*I fragment indicated on Fig 2. Nucleotides 1 to 38 represent the Jh4 coding region underlined on the coding strand.[6] Nucleotides 39 to 63 are a putative N region. The sequence from position 64 to 668 is that of the germline IL-3 gene.[28] The IL-3 TATA box (486), transcription start (515), and initiation methionine (567) are underlined. Two proposed regulatory sequences in the promotor are marked by asterisks (positions 182 and 389). (B) Comparative sequence of the t(5;14)(q31;q32) breakpoint region. The IgJh4 region is shown with its coding region, heptamer, and nonamer underlined. Clone no. 4 is shown with putative N region sequences underlined. The IL-3 sequence is also shown. A plus sign (+) denotes the identical nucleotide between sequences. No heptamer or nonamer is identified in the IL-3 sequence.

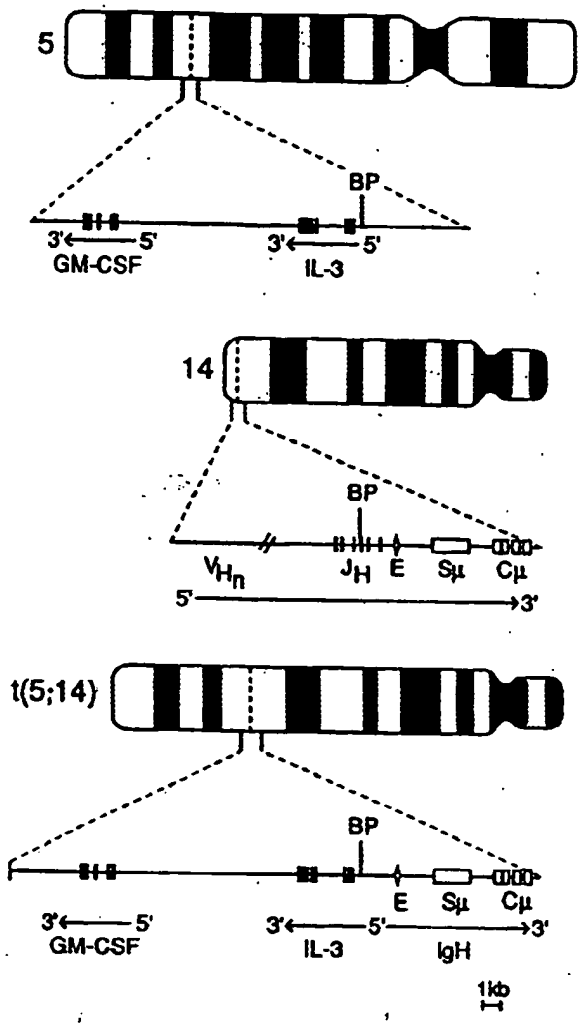

Fig 4. Diagram of the translocation. The normal chromosome 5q31 is shown with the GM-CSF gene telomeric to the IL-3 gene in the transcriptional orientation shown. On normal chromosome 14q32 the Vh regions are telomeric. The t(5;14)(q31;q32) translocation results in the head-to-head orientation of these genes. Symbols are defined in Fig 2. BP, breakpoint position.

intact as no deletions, insertions, or point mutations were detected by restriction mapping of the entire gene and sequencing of part of the gene. The IgH gene has been truncated at the Jh4 region, which places the immunoglobulin enhancer within 2.5 kb of the IL-3 gene.[17,18] This leads to the hypothesis that the enhancer is increasing transcription of a structurally normal IL-3 gene. The same mechanism is important for activation of the *c-myc* gene in some cases of Burkitt's lymphoma.[19] An alternate hypothesis is that the elimination of an upstream IL-3 promotor element is crucial to the activation of the IL-3 gene.

The proposed activation of the IL-3 gene suggests that an autocrine loop is important for the pathogenesis of this leukemia.[20] Over-expression of the IL-3 gene coupled with the presence of the IL-3 receptor in these cells could account for a strong stimulus for proliferation. In this regard, there are data indicating that immature B-lineage lymphocytes and B-lineage leukemias may express the IL-3 receptor.[21,22]

An additional feature of this type of leukemia is the dramatic eosinophilia, consisting of mature forms. It has been hypothesized that the eosinophils do not arise from the malignant clone, but are stimulated by the tumor.[23,24] Because of the known effect of IL-3 on eosinophil differentiation, secretion of high levels of IL-3 by leukemic cells might have a role in the eosinophilia in this type of leukemia.[12]

The data suggest that the recombination mechanism that is active in the IgH gene during normal differentiation has a role in this translocation.[13,14] This is supported by the breakpoint location at the 5' end of Jh4 and the presence of putative N-region sequences. On the other hand, no recombination signal sequence (heptamer and nonamer) was found in this region on chromosome 5, suggesting that additional factors also played a role. Further studies will elucidate the mechanism of this and other translocations.

In the leukemia we studied, it is possible that the immunoglobulin enhancer also activates the GM-CSF gene, since this gene is probably positioned only 14 kb away (Fig 4). This is known to be within the range of enhancer activation.[25] The interleukin-5 (IL-5) gene maps to chromosome 5q31.[26] Deregulation of the IL-5 gene by this translocation would act synergistically with IL-3 in the stimulation of eosinophil proliferation and differentiation.[27] These and other questions will be answered by the study of more patient samples. We plan to determine whether the t(5;14)(q31;q32) translocation is capable of activating multiple lymphokines simultaneously and whether they cooperate in the generation of this leukemia.

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RAPID COMMUNICATION

Activation of the Interleukin-3 Gene by Chromosome Translocation in Acute Lymphocytic Leukemia With Eosinophilia

By Timothy C. Meeker, Dan Hardy, Cheryl Willman, Thomas Hogan, and John Abrams

The t(5;14)(q31;q32) translocation from B-lineage acute lymphocytic leukemia with eosinophilia has been cloned from two leukemia samples. In both cases, this translocation joined the IgH gene and the interleukin-3 (IL-3) gene. In one patient, excess IL-3 mRNA was produced by the leukemic cells. In the second patient, serum IL-3 levels were measured and shown to correlate with disease activity. There was no evidence of excess granulocyte/macrophage colony stimulating factor (GM-CSF) or IL-5 expression. Our data support the formulation that this subtype of leukemia may arise in part because of a chromosome translocation that activates the IL-3 gene, resulting in autocrine and paracrine growth effects.

A NUMBER OF chromosome translocations have been associated with human leukemia and lymphoma. In many cases the study of these translocations has led to the discovery or characterization of proto-oncogenes, such as *bcl-2*, *c-abl*, and *c-myc*, that are located adjacent to the translocation.[1,2] It is now widely understood that cancer-associated translocations disrupt nearby proto-oncogenes.

A distinct subtype of acute leukemia is characterized by the triad of B-lineage immunophenotype, eosinophilia, and the t(5;14)(q31;q32) translocation.[3,4] Leukemic cells from such patients have been positive for terminal deoxynucleotidyl transferase (Tdt), common acute lymphoblastic leukemia antigen (CALLA), and CD19, but negative for surface or cytoplasmic immunoglobulin. In previous work, we cloned the t(5;14) breakpoint from one leukemic sample (Case 1) and determined that the IgH and interleukin-3 (IL-3) genes were joined by this abnormality.[5] In this report, we extend those findings by showing that the t(5;14)(q31;q32) translocation from a second leukemia sample (Case 2) has a similar structure, and we report our study of growth factor expression in these patients.

MATERIALS AND METHODS

Samples and Southern blots. Case 1 has been described.[5,6] Clinical features of Case 2 have been described in detail.[3] DNA isolation and Southern blotting was done using previously described methods.[5] Filters were hybridized with an immunoglobulin Jh probe, a 280 bp *Bam*HI/*Eco*RI genomic IL-3 fragment, and an IL-3 cDNA probe.[7,8]

Northern blots. RNA isolation and Northern blotting have been described.[9] Briefly, Northern blots were done by separating 9μg total RNA on 1% agarose-formaldehyde gels. Equal RNA loading in each lane was confirmed by ethidium bromide staining. Blots were hybridized with an IL-3 cDNA probe extending to the *Xho* I site in exon 5, a 720 bp *Sst* I/*Kpn* I probe derived from intron 2 of the IL-3 gene, a 600 bp *Nhe* I/*Hpa* I IL-5 cDNA probe, and a 500 bp *Pst* I/*Nco* I granulocyte-macrophage colony stimulating factor (GM-CSF) cDNA probe.[10-12]

Polymerase chain reaction. Primers were designed with *Bam*HI sites for cloning. One primer hybridized to the Jh sequences from the IgH gene (Primer 144:5'-TAGGATCCGACGGTGACCAGGGT), and the other hybridized to the region of the TATA box in the IL-3 gene (Primer 161: 5'-AACAGGATCCCGCCTTATATGTGCAG). Polymerase chain reaction (PCR) (95°C for 1 minute, 61°C for 30 seconds, and 72°C for 3 minutes) was done using 500 ng genomic DNA and 50 pmol of each primer in 100 μL containing 67 mmol/L Tris-HCl pH 8.8, 6.7 mmol/L $MgCl_2$, 10% dimethyl sulfoxide (DMSO), 170 μg/mL bovine serum albumin (BSA) (fraction V), 16.6 mmol/L ammonium sulfate, 1.5 mmol/L each dNTP and Taq polymerase (Perkin-Elmer, Norwalk, CT).[13]

Sequencing. Sequencing was done by chain termination in M13 vectors.[14] As part of this study, we sequenced a subclone of a normal IL-3 promotor, covering 598 base pairs from a *Sma* I site at position −1240 (with respect to the proposed site of transcription initiation) to an *Nhe* I site at position −642. The plasmid containing this region was a gift from Naoko Arai of the DNAX Research Institute.

Expression in Cos7 cells. A genomic IL-3 fragment from Case 1 was cloned into the pXM expression vector.[15] Briefly, the *Hin*dIII/*Sal* I fragment containing the IL-3 gene was subcloned from the previously described phage clone 4 into pUC18.[5] The 2.6 kb fragment extending from the *Sma* I site 61 bp upstream of the IL-3 transcription start to the *Sma* I site in the polylinker was cloned into the blunted *Xho* I site of pXM. The negative control construct was the pXM vector without insert. Plasmids were introduced into Cos7 cells by electroporation, and supernatant was collected after 48 hours in culture.

TF1 bioassay. TF-1 cells were passaged in RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, 2 mmol L-glutamine, and 1 ng/mL human GM-CSF.[15] Samples and antibodies were diluted in this same medium lacking GM-CSF but containing penicillin and streptomycin. A 25 μL volume of serial dilutions of patient serum was added to wells in a flat bottom 96-well microtiter plate. Rat anti-cytokine monoclonal antibody in a volume of 25 μL was added to appropriate wells and preincubated for 1 hour at 37°C. Fifty microliters of twice washed TF-1 cells were added to each well, giving a final cell concentration of 1×10^4 cells per well (final volume, 100 μL). The plate was incubated for 48 hours. The remaining cell viability was determined metabolically by the colori-

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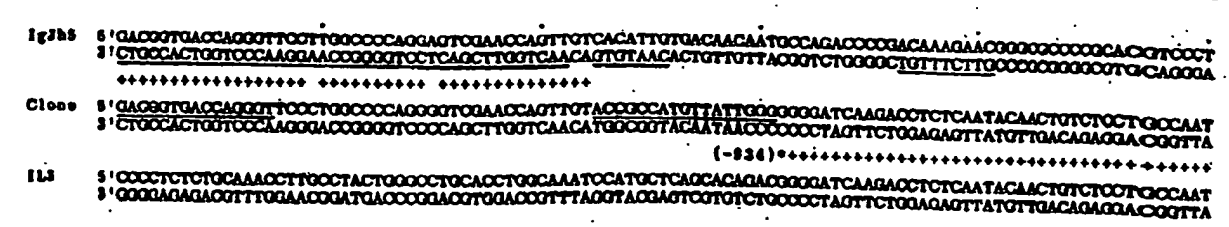

Fig 1. Breakpoint sequences for Case 2. The germline IgJh5 region sequence (protein coding region and recombination signal sequences are underlined) is on top, the translocation sequence from Case 2 (PCR primer sequences and putative N region are underlined) is in the middle, and the germline IL-3 sequence, which we derived from a normal IL-3 clone, is on the bottom.' + indicates that each sequence has the same nucleotide. The sequence documents the head-to-head joining of the IL-3 and IgH genes. The breakpoint in the IL-3 gene occurred at position −934 (*).

metric method of Mosmann using a VMax microtiter plate reader (Molecular Devices, Menlo Park, CA) set at 570 and 650 nm.[16]

Cytokine Immunoassays. These assays used rat monoclonal anti-cytokine antibodies (10 μg/mL) to coat the wells of a PVC microtiter plate. The capture antibodies used were BVD3-6G8, JES1-39D10, and BVD2-23B6, for the IL-3, IL-5, and GM-CSF assays, respectively. Patient sera were then added (undiluted and diluted 1:2 for IL-3, undiluted for IL-5, and undiluted and diluted 1:5 for GM-CSF). The detecting immunoreagents used were either mouse antiserum to IL-3 or nitroiodophenyl (NIP)-derivatized rat monoclonal antibodies JES1-5A2 and BVD2-21C11, specific for IL-5 and GM-CSF, respectively. Bound antibody was subsequently detected with immunoperoxidase conjugates: horseradish peroxidase (HRP)-labeled goat anti-mouse Ig for IL-3, or HRP-labeled rat (J4 MoAb) anti-NIP for IL-5 and GM-CSF. The chromogenic substrate was 3-3'azino-bis-benzthiazoline sulfonate (ABTS; Sigma, St Louis, MO). Unknown values were interpolated from standard curves prepared from dilutions of the recombinant factors using Softmax software available with the VMAX microplate reader (Molecular Devices).

RESULTS

Leukemic DNA from Case 2 was studied by Southern blotting. When digested with the *Hin*dIII restriction enzyme and hybridized with a human immunoglobulin heavy chain joining region (Jh) probe, a rearranged fragment at approximately 14 kb was detected (data not shown). When reprobed with either of two different IL-3 probes, a rearranged 14 kb fragment, comigrating with the rearranged Jh fragment, was identified. When leukemic DNA was digested with *Hin*dIII plus *Eco*RI, a rearranged Jh fragment was detected at 6 kb. The IL-3 probes also identified a comigrating fragment of this size. These experiments indicated that the leukemic sample studied was clonal and that a single fragment contained both Jh and IL-3 sequences, suggesting a translocation had occurred.

To characterize better the joining of the IL-3 gene and the immunoglobulin heavy chain (IgH) gene, the polymerase chain reaction (PCR) was used to clone the translocation.[13] A Jh primer and an IL-3 primer were designed to produce an amplified product in the event of a head-to-head translocation. While control DNA gave no PCR product, Case 2 DNA yielded a PCR-derived fragment of approximately 980 bp, which was cloned and sequenced.

The DNA sequence of the translocation clone from Case 2 confirmed the joining of the Jh region with the promotor of the IL-3 gene in a head-to-head configuration (Fig 1). Sequence analysis indicated that the breakpoint on chromosome 14 was just upstream of the Jh5 coding region. The breakpoint on chromosome 5 occurred 934 bp upstream of the putative site of transcription initiation of the IL-3 gene. We also determined that a putative N sequence of 17 bp was inserted between the chromosome 5 and chromosome 14 sequences during the translocation event.[17,18] Figure 2 shows

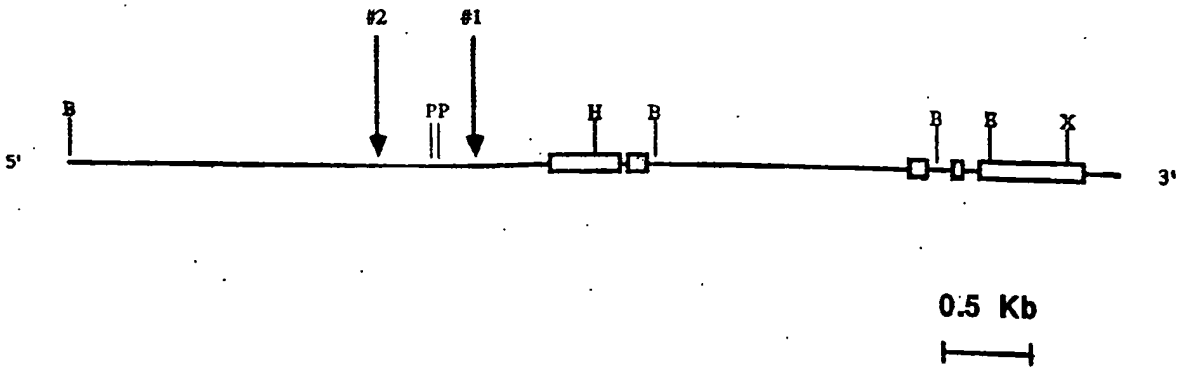

Fig 2. Relationship of chromosome 5 breakpoints to the IL-3 gene. This figure shows the two cloned breakpoints (arrows) in relation to the normal IL-3 gene.[8,10] One breakpoint occurred at position −462 and the other at −934 (arrows). In both circumstances, the translocations resulted in a head-to-head joining of the IgH gene and the IL-3 gene, leaving the mRNA and protein coding regions of the IL-3 gene intact. Boxes denote the five IL-3 exons; restriction enzymes are (B) *Bam*HI, (P) *Pst* I, (H) *Hpa* I, (E) *Eco*RI, and (X) *Xho* I.

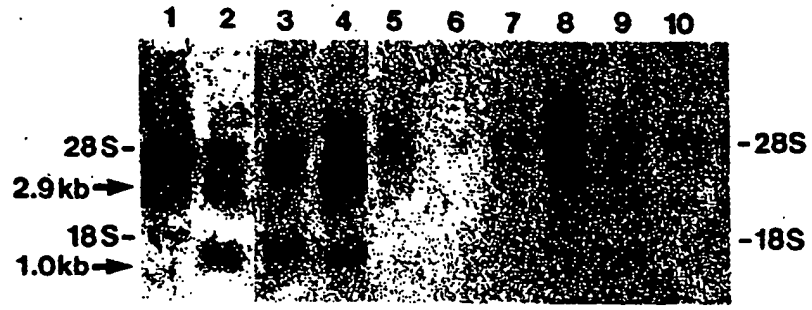

Fig 3. Documentation of IL-3 mRNA over-expression. A Northern blot was prepared and hybridized with a probe for IL-3. Lane 1 contained RNA from unstimulated peripheral blood lymphocytes (PBL) as a negative control. Lane 2 contained RNA from PBL stimulated for 4 hours with concanavalin A (ConA), and lane 3 contained RNA from PBL stimulated with ConA for 48 hours. As in the positive control lanes (2 and 3), a 1 kb band was identified in the leukemic sample from Case 1 (lane 4, lower arrow), suggesting aberrant expression of the IL-3 gene. In addition, the leukemic sample showed over-expression of an unspliced 2.9 kb IL-3 transcript (lane 4, upper arrow). We documented that this represented an unspliced precursor of the mature 1 kb transcript by showing that this band hybridized to a probe from intron 2 of the IL-3 gene. A similar 2.9 kb band was detected in lane 2, suggesting that an IL-3 mRNA of this size is sometimes detectable in normal mitogen-stimulated cells. Lane 5 through 10 represent RNA from six samples of B-lineage acute lymphocytic leukemia without the t(5;14) translocation, indicating that only the sample with the translocation exhibited IL-3 over-expression. Case 2 could not be analyzed by Northern blot because too few cells were available for study.

the locations of the two cloned breakpoints in relation to the IL-3 gene. The two chromosome 5 breakpoints were separated by less than 500 bp.

The genomic structure in Cases 1 and 2 suggested that a normal IL-3 gene product was over-expressed as a result of the altered promotor structure. This would predict that the IL-3 gene on the translocated chromosome was capable of making IL-3 protein. This prediction was tested by expressing a genomic fragment from the translocated allele of Case 1 containing all five IL-3 exons under the control of the SV40 promotor/enhancer in the Cos7 cell line. Cell supernatants were studied in a proliferation assay using the factor dependent erythroleukemic cell line, TF-1. The supernatants derived from transfections using the vector plus insert supported TF-1 proliferation, while supernatants from transfections using the vector alone were negative in this assay (data not shown). Furthermore, the biologic activity could be blocked by an antibody to human IL-3 (BVD3-6G8). This result showed that the translocated allele retained the ability to make IL-3 mRNA and protein.

The level of expression of IL-3 mRNA in leukemic cells from Case 1 was assessed. Northern blotting showed that the mature IL-3 mRNA (approximately 1 kb) and a 2.9 kb unspliced IL-3 mRNA were excessively produced by the leukemia (Fig 3). The 2.9 kb form of the mRNA is also present at low levels in normal peripheral blood T lymphocytes after mitogen activation (Fig 3). Several B-lineage acute leukemia samples without the t(5;14) translocation had undetectable levels of IL-3 mRNA in these experiments. In addition, although genes for GM-CSF and IL-5 map close to the IL-3 gene and might have been deregulated by the translocation, no IL-5 or GM-CSF mRNA could be detected in the leukemic sample (data not shown).[19,20]

Three serum samples from Case 2 were assayed by immunoassay for levels of IL-3, GM-CSF, and IL-5 (Table 1). Serum IL-3 could be detected and correlated with the clinical course. When the patient's leukemic cell burden was highest, the IL-3 level was highest. No serum GM-CSF or IL-5 could be detected.

Since the IL-3 immunoassay measured only immunoreactive factor, we confirmed that biologically active IL-3 was present by using the TF-1 bioassay. This bioassay can be rendered monospecific using appropriate neutralizing monoclonal antibodies specific for IL-3, IL-5, or GM-CSF. We observed that sera from 1-16-84 and 3-14-84 contained TF-1 stimulating activity that could be blocked with anti-IL-3 MoAb (BVD3-6G8), but not with MoAbs to IL-5 (JES1-39D10) or GM-CSF (BVD2-23B6) (Fig 4; GM-CSF data not shown). The amount of neutralizable bioactivity in these two samples correlated very well with the difference in IL-3 levels obtained by immunoassay for these samples. Furthermore, the failure to block TF-1 proliferating activity with either anti-IL-5 or anti-GM-CSF was consistent with the inability to measure these factors by immunoassay and

Table 1. Peripheral Blood Counts and Growth Factor Levels at Different Times in Case 2

	Sample Date		
	11/15/83	1/16/84	3/14/84
Peripheral blood counts (cells/μL)			
WBC	81,800	116,500	12,300
Lymphoblasts	0	33,785	0
Eosinophils	46,626	73,080	615
Serum growth factor levels (pg/mL)			
IL-3	<444	7,995	1,051
GM-CSF	<15	<15	<15
IL-5	<50	<50	<50

Peripheral blood counts from Case 2 at three different time points with the corresponding growth factor levels quantified by immunoassay. The patient received chemotherapy between 1/16/84 and 3/14/84 to lower his leukemic burden.[3] No serum samples were available for a similar analysis of Case 1.

Abbreviation: WBC, white blood cells.

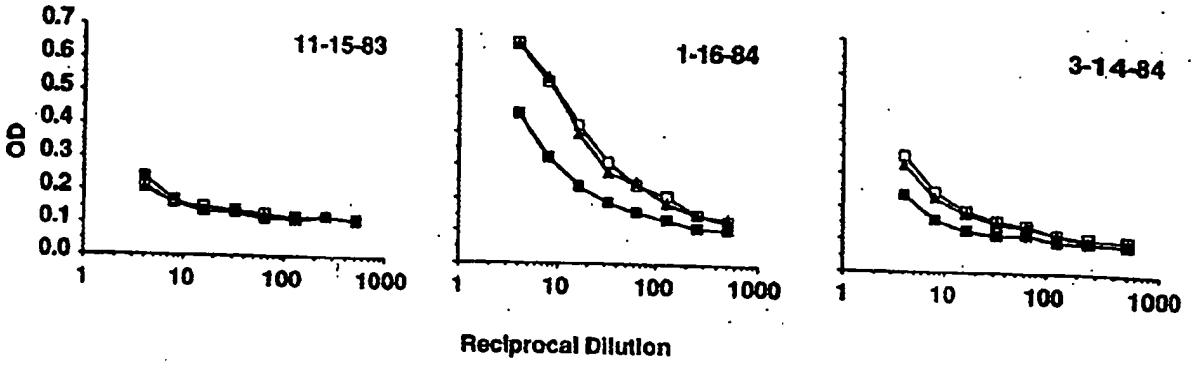

Fig 4. Bioassay of serum IL-3. Leukemic patient sera were tested for bioactive IL-3 and IL-5 in the TF-1 proliferation assay. The reciprocal of the dilution is indicated on the horizontal axis and the optical density indicating the amount of proliferation is indicated on the vertical axis. Serum from all three time points was assayed simultaneously. The assay was rendered monospecific by using a 1 μg/mL final concentration of monoclonal rat anti–IL-3, BVD3-6G8 (■), or anti–IL-5, JES1-39D10 (▲); □ indicates no MoAb. On 1/16/84 and 3/14/84, inhibition of proliferation was evident in the presence of anti–IL-3 antibody, documenting serum levels of IL-3 on those days. Serum IL-5 was not detected in this assay, as anti–IL-5 did not alter TF-1 proliferation.

indicated that these other myeloid growth factors were not detectably circulating in the serum of this patient.

DISCUSSION

In this report, we have extended our analysis of acute lymphocytic leukemia and eosinophilia associated with the t(5;14) translocation. In both cases we have studied, we have documented the joining of the IL-3 gene from chromosome 5 to the IgH gene from chromosome 14. The breakpoints on chromosome 5 are within 500 bp of each other, suggesting that additional breakpoints will be clustered in a small region of the IL-3 promotor. The PCR assay we have developed will be useful in the screening of additional clinical samples for this abnormality.

The finding of a disrupted IL-3 promotor associated with an otherwise normal IL-3 gene implied that this translocation might lead to the over-expression of a normal IL-3 gene product. In this work, we have documented that this is true. In addition, neither GM-CSF nor IL-5 are over-expressed by the leukemic cells. Furthermore, in one patient, serum IL-3 could be measured and correlated with disease activity. To our knowledge, this is the first measurement of human IL-3 in serum and its association with a disease process. The measurement of serum IL-3 in this and other clinical settings may now be indicated.

The finding of the IL-3 gene adjacent to a cancer-associated translocation breakpoint suggests that its activation is important for oncogenesis. It is our thesis that an autocrine loop for IL-3 is important for the evolution of this leukemia.[21] The excessive IL-3 production that we have documented would be one feature of such an autocrine loop. The final proof of our thesis must await additional data. In particular, from the study of additional clinical samples, it will be necessary to document that the IL-3 receptor is present on the leukemic cells and that anti–IL-3 antibody decreases proliferation of the leukemia in vitro.

An important aspect of this work is the suggestion of a therapeutic approach for this disease. If an autocrine loop for IL-3 can be documented in this disease, attempts to lower circulating IL-3 levels or block the interaction of IL-3 with its receptor may prove useful. Because it is also possible that the eosinophilia in these patients is mediated by the paracrine effects of leukemia-derived IL-3, similar interventions may improve this aspect of the disease. Antibodies or engineered ligands to accomplish these goals may soon be available.

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Clinical and Pathologic Significance of the c-*erb*B-2 (*HER*-2/*neu*) Oncogene

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The c-*erb*B-2 oncogene was first shown to have clinical significance in 1987 by Slamon et al,[79] who reported that c-*erb*B-2 DNA amplification in breast carcinomas correlated with decreased survival in patients with metastasis to axillary lymph nodes. Subsequent studies, however, of c-*erb*B-2 activation in breast carcinoma reached conflicting conclusions about its clinical significance. This oncogene also has been reported to have clinical and pathologic implications in other neoplasms. Our review summarizes these various studies and examines the clinical relevance of c-*erb*B-2 activation, which has not been emphasized in recent reviews.[37,38,55] The molecular biology of the c-*erb*B-2 oncogene has been extensively reviewed[37,38,55] and will be discussed only briefly here.

BACKGROUND

The c-*erb*B-2 oncogene was discovered in the 1980s by three lines of investigation. The *neu* oncogene was detected as a mutated transforming gene in neuroblastomas induced by ethylnitrosurea treatment of fetal rats.[8,73,74,75] The c-*erb*B-2 was a human gene discovered by its homology to the retroviral gene v-*erb*B.[33,49,76] *HER*-2 was isolated by screening a human genomic DNA library for homology with v-*erb*B.[84] When the DNA sequences were determined subsequently, c-*erb*B-2, *HER*-2, and *neu* were found to represent the same gene. Recently, the c-*erb*B-2 oncogene also has been referred to as *NGL*.

The c-*erb*B-2 DNA is located on human chromosome 17q21[24,33,68] and codes for c-*erb*B-2 mRNA (4.6 kb), which translates c-*erb*B-2 protein (p185). This

protein is a normal component of cytoplasmic membranes. The c-*erb*B-2 oncogene is homologous with, but not identical to, c-*erb*B-1, which is located on chromosome 7 and codes for the epidermal growth factor receptor.[8,103] The c-*erb*B-2 protein is a receptor on cell membranes and has intracellular tyrosine kinase activity and an extracellular binding domain.[8,105] Electron microscopy with a polyclonal antibody detects c-*erb*B-2 immunoreactivity on cytoplasmic membranes of neoplasms, especially on microvilli and the non-villous outer cell membrane.[61] In normal cells, immunohistochemical reactivity for c-*erb*B-2 is frequently present at the basolateral membrane or the cytoplasmic membrane's brush border.[52,62]

There is experimental evidence that c-*erb*B-2 protein may be involved in the pathogenesis of breast neoplasia. Overproduction of otherwise normal c-*erb*B-2 protein can transform a cell line into a malignant phenotype.[25] Also, when the *neu* oncogene containing an activating point mutation is placed in transgenic mice with a strong promoter for increased expression, the mice develop multiple independent mammary adenocarcinomas.[13,63] In other experiments, monoclonal antibodies against the *neu* protein inhibit the growth (in nude mice) of a *neu*-transformed cell line,[24–25] and immunization of mice with *neu* protein protects them from subsequent tumor challenge with the *neu*-transformed cell line.[14] Some authors have speculated that the use of antagonists for the unknown ligand could be useful in future chemotherapy.[25] Further review of this experimental evidence is beyond the scope of this article.

The c-*erb*B-2 activation most likely occurs at an early stage of neoplastic development. This hypothesis is supported by the presence of c-*erb*B-2 activation in both in situ and invasive breast carcinomas. In addition, studies of metastatic breast carcinomas usually demonstrate uniform c-*erb*B-2 activation at multiple sites in the same patient,[11,12,30,41,82] although c-*erb*B-2 activation has rarely been detected in metastatic lesions but not in the primary tumor.[57,59,107] Even more rarely, c-*erb*B-2 DNA amplification has been detected in a primary breast carcinoma but not in its lymph node metastasis.[5] In patients who have bilateral breast neoplasms, both lesions have similar patterns of c-*erb*B-2 activation, but only a few such cases have been studied.[11]

MECHANISMS OF c-*erb*B-2 ACTIVATION

The most common mechanism of c-*erb*B-2 activation is genomic DNA amplification, which almost always results in overproduction of c-*erb*B-2 mRNA and protein.[17,24,65,81] The c-*erb*B-2 amplification may stabilize the overproduction of mRNA or protein through unknown mechanisms. Human breast carcinomas with c-*erb*B-2 amplification contain 2 to 40 times more c-*erb*B-2 DNA[4,8] and 4 to 128 times more c-*erb*B-2 mRNA[24,90] than found in normal tissue. Most human breast carcinomas with c-*erb*B-2 amplification have 2 to 15 times more c-*erb*B-2 DNA. Tumors with greater amplification tend to have greater overproduction.[17,52,65] The non-mammary neoplasms that have been studied tend to have

similar levels of c-*erb*B-2 amplification or overproduction relative to the corresponding normal tissue.

The second most common mechanism of c-*erb*B-2 activation is overproduction of c-*erb*B-2 mRNA and protein without amplification of c-*erb*B-2 DNA.[51] The quantities of mRNA and protein usually are less than those in amplified cases and may approach the small quantities present in normal breast or other tissues.[17,50,52] The c-*erb*B-2 protein overproduction without mRNA overproduction or DNA amplification has been described in a few human breast carcinoma cell lines.[47]

Other rare mechanisms of c-*erb*B-2 activation have been reported. Translocations involving the c-*erb*B-2 gene have been described in a few mammary and gastric carcinomas, although some reported cases may represent restriction fragment length polymorphisms or incomplete restriction enzyme digestions that mimic translocations.[31,65,75,84,90,108] A single point mutation in the transmembrane portion of *neu* has been described in rat neuroblastomas induced by ethylnitrosurea.[9,55] The mutated *neu* protein has increased tyrosine kinase activity and aggregates at the cell membrane.[10,83,98] Although there has been speculation that some of the amplified c-*erb*B-2 genes may contain point mutations,[46] none has been detected in primary human neoplasms.[41,53,61]

TECHNIQUES FOR DETECTING c-*erb*B-2 ACTIVATION

Detection of c-*erb*B-2 DNA Amplification

Amplification of c-*erb*B-2 DNA is usually detected by DNA dot blot or Southern blot hybridization. In the dot blot method, the extracted DNA is placed directly on a nylon membrane and hybridized with a c-*erb*B-2 DNA probe. In the Southern blot method, the extracted DNA is treated with a restriction enzyme, and the fragments are separated by electrophoresis, transferred to a nylon membrane, and hybridized with a c-*erb*B-2 DNA probe. In both techniques, c-*erb*B-2 amplification is quantified by comparing the intensity (measured by densitometry) of the hybridization bands from the sample with those from control tissue.

Several technical problems may complicate the measurement of c-*erb*B-2 DNA amplification. First, the extracted tumor DNA may be excessively degraded or diluted by DNA from stromal cells.[81] Second, the c-*erb*B-2 DNA probe must be carefully chosen and labeled. For example, oligonucleotide c-*erb*B-2 probes may not be sensitive enough for measuring a low level of c-*erb*B-2 amplification, because diploid copy numbers can be difficult to detect (unpublished data). Third, the total amounts of DNA in the sample and control tissue must be compensated for, often with a probe to an unamplified gene. Many studies have used control probes to genes on chromosome 17, the location of c-*erb*B-2, to correct for possible alterations in chromosome number. Identical results, however, are obtained by using control probes to genes on other chromosomes,[5,66,80] with rare exception.[17] Studies using control probes to the beta-

globin gene must be interpreted with caution, because one allele of this gene is deleted occasionally in breast carcinomas.[3]

Amplification of c-*erb*B-2 DNA was assessed by using the polymerase chain reaction (PCR). In one recent study,[32] Oligoprimers for the c-*erb*B-2 gene and a control gene are added to the sample's DNA, and PCR is performed. If the sample contains more copies of c-*erb*B-2 DNA than of the control gene, the c-*erb*B-2 DNA is replicated preferentially.

Detection of c-*erb*B-2 mRNA Overproduction

Overproduction of c-*erb*B-2 mRNA usually is measured by RNA dot blot or Northern blot hybridization. Both techniques require extraction of RNA but otherwise are analogous to DNA dot blot and Southern blot hybridization. Use of PCR for detection of c-*erb*B-2 mRNA has been described in two recent abstracts.[88,102]

Overproduction of c-*erb*B-2 mRNA can be measured by in situ hybridization. Sections are mounted on glass slides, treated with protease, hybridized with a radiolabeled probe, washed, treated with nuclease to remove unbound probe, and developed for autoradiography. Silver grains are seen only over tumor cells that overproduce c-*erb*B-2 mRNA. Negative control probes are used.[65,88,105] Our experience indicates that these techniques are relatively insensitive for detecting c-*erb*B-2 mRNA overproduction in routinely processed tissue. Although the sensitivity may be increased by modifications that allow simultaneous detection of c-*erb*B-2 DNA and mRNA, in situ hybridization still is cumbersome and expensive (unpublished data).

All of the above c-*erb*B-2 mRNA detection techniques have several problems that make them more difficult to perform than techniques for detecting DNA amplification. One major problem is the rapid degradation of RNA in tissue that is not immediately frozen or fixed. In addition, during the detection procedure, RNA can be degraded by RNase, a ubiquitous enzyme, which must be eliminated meticulously from laboratory solutions. Third, control probes to genes that are uniformly expressed in the tissue of interest need to be carefully selected.

Detection of c-*erb*B-2 Protein Overproduction

The most accurate methods for detecting c-*erb*B-2 protein overproduction are the Western blot method and immunoprecipitation. Both techniques can document the binding specificity of various antibodies against c-*erb*B-2 protein. In Western blot studies, protein is extracted from the tissue, separated by electrophoresis (according to size), transferred to a membrane, and detected by using antibodies to c-*erb*B-2. In immunoprecipitation studies, antibodies against c-*erb*B-2 are added to a tumor lysate, and the resulting protein–antibody precipitate is separated by gel electrophoresis and stained for protein. Both Western blot and immunoprecipitation are useful research tools but currently are not practical for diagnostic pathology. Two recent abstracts have described an enzyme-linked immunosorbent assay (ELISA) for detection of c-*erb*B-2 protein.[18,45]

Overproduction of c-erbB-2 protein is most commonly assessed by various immunohistochemical techniques. These procedures often generate conflicting results, which are explained at least partially by three factors. First, various studies have used different polyclonal and monoclonal antibodies. Because some polyclonal antibodies recognize weak bands in addition to the c-*erb*B-2 protein band on Western blot or immunoprecipitation, the results of these studies should be interpreted with caution.[23,35,47,61] Even some monoclonal antibodies immunoprecipitate protein bands in addition to c-*erb*B-2 (p185).[30,59,66] Second, tissue fixation contributes to variability between studies. For example, some antibodies detect c-erbB-2 protein only in frozen tissue and do not react in fixed tissue. In general, formalin fixation diminishes the sensitivity of immunohistochemical methods and decreases the number of reactive cells.[41,59] When Bouin's fixative is used, there may be a higher percentage of positive cases.[22] Third, minimal criteria for interpreting immunohistochemical staining are generally lacking. Although there is general agreement that distinct crisp cytoplasmic membrane staining is diagnostic for c-*erb*B-2 activation in breast carcinoma, the number of positive cells and the staining intensity required to diagnose c-*erb*B-2 protein overproduction varies from study to study and from antibody to antibody. Detection of c-*erb*B-2 protein is not a problem because it can be detected in tissue form more than 24 hours after tumor resection without fixation or freezing.

ACTIVATION OF c-*erb*B-2 IN BREAST LESIONS

Incidence of c-*erb*B-2 Activation

Most studies of c-*erb*B-2 gene activation do not specify histological subtypes of infiltrating breast carcinoma. Amplification of c-*erb*B-2 DNA was found in 19.1 percent (519 of 2714) of invasive carcinomas in 25 studies (Table 1), and c-*erb*B-2 mRNA or protein overproduction was detected in 20.9 percent (566 of 2714) of invasive carcinomas in 20 studies. Twelve studies have documented c-*erb*B-2 mRNA or protein overproduction in 15 percent (88 of 604) of carcinomas that lacked c-*erb*B-2 DNA amplification.

The incidence of c-*erb*B-2 activation in infiltruting breast carcinoma varies with the histological subtype. Approximately 22 percent (142 of 650) of infiltrating ductal carcinomas have c-*erb*B-2 activation, as expected from the above data. Other variants of invasive carcinoma with frequent c-*erb*B-2 activation are inflammatory carcinoma (74 percent, 54 of 87), Paget's disease (82 percent, 9 of 11), and medullary carcinoma (22 percent, 5 of 23). In contrast, c-*erb*B-2 activation is infrequent in invasive lobular carcinoma (7 percent, 5 of 73) and tubular carcinoma (7 percent, 1 of 15).

The c-*erb*B-2 protein overproduction is present in 44 percent (44 of 100) of ductal carcinomas in situ, especially comedocarcinoma in situ (68 percent, 49 of 72). The micropapillary variant of ductal carcinoma in situ also tends to have c-*erb*B-2 activation,[40,54] especially if larger cells are present. The greater fre-

TABLE 1. c-*erb*B-2 ACTIVATION IN MALIGNANT HUMAN BREAST NEOPLASMS

Histological Diagnosis	c-*erb*B-2 DNA Amplification[a]	c-*erb*B-2 mRNA Overproduction	c-*erb*B-2 Protein Overproduction[b]
	19/103,[79] 15/95,[83] 15/86,[111] 17/73,[77] 16/66,[42] 6/61,[83] 11/57,[52] 10/57,[45] 13/51,[13] 8/48,[81] 10/38,[82] 12/36,[84] 1/25,[13] 7/24,[91] 7/15,[31] 7/10,[88] 2/10[107]		24/53,[82] [illegible] 22/45,[8] 11/36,[84] 7/24,[91] 1/10[81]
Carcinoma, type not specified but lacking c-*erb*B-2 DNA amplification	—	18/136,[81] 14/73,[34] 8/16,[85] 0/8,[80] 1/4,[31] 0/3[86]	16/231,[170] 18/136,[81] 13/35,[13] 14/29,[82b] 1/28,[82] 3/24,[84] 0/17[91]
Infiltrating ductal carcinoma	21/116,[82] 23/107,[84] 17/50,[44] 7/37[80] 14/53 (comedo-carcinoma)[18] 3/33 (tubuloductal carcinoma)[16]	35/85[34]	22/137,[40] 14/83,[89] 9/34[88]

Inflammatory carcinoma	33/80,[38] 3/6[52]	46/75[38]	5/5[220]
Paget's disease	—	—	5/6,[49] 2/3,[54] 2/2[62]
Tubular carcinoma	0/5,[18] 0/1[33]	—	1/9[40]
Medullary carcinoma	2/4,[18] 0/1[34]	0/1[34]	1/12,[40] 1/3,[68] 1/2,[62] 0/1[39]
Mucinous carcinoma	0/1,[18] 0/1[62]	—	1/2[68]
Invasive papillary carcinoma	0/2[62]	—	—
Infiltrating lobular carcinoma	1/15,[18] 0/6[34]	1/5[34]	2/27,[62] 0/12,[40] 0/9,[68] 1/5[68]
Mammary fibromatosis	0/1[50]	—	—
Ductal CIS	0/2[54]	1/2[54]	[illegible]
Ductal CIS, solid or comedo type	—	—	20/33,[54] 19/29,[62] 10/10[54]
Ductal CIS, micropapillary type	—	—	10/10[40]
Ductal CIS, micropapillary or cribriform type	—	—	1(local)/14[54]
Ductal CIS, papillary or cribriform type	—	—	0/16,[62] 1/9,[68] 0/3[40]
Lobular CIS	—	—	0/16[40]

[a]Shown as number of cases with activation/number of cases studied; reference is given as a superscript.
[b]These protein studies used Western blots; the rest used immunohistochemical methods.
[c]CIS = carcinoma in situ.

quency of c-*erb*B-2 protein [illegible] oduction in comedocarcinoma in situ, compared with infiltrating duct[illegible] noma, could be explained by the fact that many infiltrating ductal carc[illegible] arise from other types of intraductal carcinoma, which show c-*erb*B-[illegible] ation infrequently. Others have speculated that carcinoma in situ with [illegible]-2 activation tends to regress or to lose c-*erb*B-2 activation during pr[illegible]on to invasion.[40,58,62] Infiltrating and in situ components of ductal carci[illegible]ever, usually are similar with respect to c-*erb*B-2 activation,[11,29] althou[illegible] authors have noted more heterogeneity of the immunohistochemical s[illegible]attern in invasive than in in situ carcinoma.[40,42,58] Activation of c-[illegible]s infrequent in lobular carcinoma in situ. If lesions contain more than [illegible]gical pattern of carcinoma in situ, the c-*erb*B-2 protein overprodu[illegible]d to occur in the comedocarcinoma in situ but may include other ar[illegible]cinoma in situ.[42,54,58] Overproduction of c-*erb*B-2 protein in ductal ca[illegible] in situ correlates with larger cell size and a periductal lymphoid infilt[illegible]

Activation of c-*erb*B-[illegible] been identified in benign breast lesions, including fibrocystic diseas[illegible]nomas, and radial scars (Table 2). Strong membrane immunohistoche[illegible]tivity for c-*erb*B-2 has not been described in atypical ductal hyperpla[illegible]ver; accentuation of membrane staining has been noted infreque[illegible] normal breast tissue, c-*erb*B-2 DNA is diploid, and c-*erb*B-2 is ex[illegible]wer levels than in activated tumors.[34,55,65,68]

These preliminary da[illegible]that c-*erb*B-2 activation may not be useful for resolving many of the c[illegible]blems in diagnostic surgical pathology. For example, c-*erb*B-2 activat[illegible]ent in tubular carcinoma and radial scars. In addition, because c-*erb*[illegible]n is unusual in atypical ductal hyperplasia, cribriform carcinoma in sit[illegible]carcinoma in situ, detection of c-*erb*B-2 activation in these lesions[illegible]d in their differential diagnosis. The histological features of co[illegible]u, which commonly overproduces c-*erb*B-2, are unlikely to [illegible]e of benign lesions. Activation of

TABLE 2. c-*erb*B-2 ACTIVAT[illegible] IN BREAST LESIONS

Histological Diagnosis	c-*erb*B-2 mRNA [illegible]production	c-*erb*B-2 Protein Overproduction
Fibrocystic disease	—	0/32,[29] 0/9,[58] 0/8[58]
Atypical ductal hyperplasia	—	2(weak)/21,[64] 1(cytoplasmic)/13[29]
Benign ductal hyperplasia	—	0/12[29]
Sclerosing adenosis	—	0/4[29]
Fibroadenomas	[illegible]0/3[34]	0/21,[29] 0/10,[58] 0/8,[58] 0/3[42]
Radial scars	—	0/22[29]
Blunt duct adenosis	—	0/14[29]
"Breast mastosis"	[illegible]	—

*Shown as number of cases with [illegible], reference is given as a superscript.

c-*erb*B-2, however, doe ... tr... etal carcinoma over infiltrating lobular carcinoma. Furt... es ... ies would be useful.

Correlation of c-*erb*B-... thologic Prognostic Factors

Multiple studies have st... -*erb*B-2 activation with various pathologic prognostic fa... ion of c-*erb*B-2 was correlated with lymph node metast... :h higher histological grade in 6 of 17 series, and with hi... ries. Large tumor size was not associated with c-*erb*B-2... s (11 of 14). Tetraploid DNA content and low prolife... m ...-67, have been suggested as prognostic factors and ... 2 activation.[6,7]

Correlation of c-*erb*B-... cal-Prognostic Factors

Various studies have atte... -*erb*B-2 activation with clinical features that may pred... le 4). Activation of c-*erb*B-2 correlated with absence... in 10 of 28 series and with absence of progesterone r... . In most studies, patient age did not correlate with ... in the rest of the reports, c-*erb*B-2 activation w... ger or older ages.

Correlation of c-*erb*B-... nt Outcome

Slamon et al[79,81] first st... the c-*erb*B-2 oncogene independently predicts ... s with breast carcinoma. The correlation of c-*erb*B-... tcome was nearly as strong as the correlation of ... s with poor outcome. Slamon et al also reported t... important prognostic indicator only in patient... [81]

A large numbe... empted to correlate c-*erb*B-2 activation with prognos... s, there was a correlation between c-*erb*B-2 a... r decreased survival. In five of these series, th... tivation was reported to be independent of o... st, 18 series did not confirm the correlation of ... nce or survival. Four possible explanations for th... ow.

One problem ... n correlates with prognosis mainly in patient... summarized in Table 5, most studies of patients with ... stasis showed a correlation of c-*erb*B-2 activatio... ast, most studies of patients without axillary ... d a correlation with patient outcome. Table ... h all ... tients (with and without axillary met... groups. There is a trend for studies with a h... ases to show an association between c-*erb*B-... . Thus, most of the current evidence sugges... gnostic value only in patients with metastasis to ...

TABLE 3. CORRELATION OF *c-erbB-2* ACTIVATION WITH PATHOLOGIC PROGNOSTIC FACTORS IN BREAST CARCINOMA

Prognostic Factor	P[a]	c-erbB-2 DNA Amplification[b]	c-erbB-2 mRNA Overproduction	c-erbB-2 Protein Overproduction[c]
		(41)[87]		
High		(47)[13] (1	(53)[11]	(176)[101] (168)[11] (38)[13]
		(58)[111] (50)[44] (41)[85]	(86)[7]	(288)[56] (189)[56] (13[illegible]

[a]A correlation is statistically significant at <0.05, equivocal at best between 0.05 and 0.15, and not statistically significant at >0.15.
[b]Numbers inside parentheses are the number of patients in an individual study; superscript is the reference. Some studies analyzed more than one group of patients.
[c]By Western blot method; all other protein studies used immunohistochemical methods.

TABLE 4. CORRELATION OF c-erbB-2 ACTIVATION WITH CLINICAL PROGNOSTIC FACTORS IN BREAST CARCINOMA

Prognostic Factor	P*	c-erbB-2 DNA Amplification[b]	c-erbB-2 mRNA Overproduction	c-erbB-2 Protein Overproduction[c]
		(Younger: 5[illegible])		
	<0.15	(238)[17] (176)[87] (157)[13]	(62)[86]	(350)[256] (290)[58] (18[illegible]
		(1[illegible] (116)[34] (10[illegible]		(162)[11] (45)[82]
		(55)[17] (34)[17] (5[illegible]		
		(56)[32] (53)[71] (49)[13]		
		(41)[33] (15)[71]		

*A correlation is statistically significant at <0.05, equivocal at best between 0.05 and 0.15, and not statistically significant at >0.15
[b]Numbers inside parentheses are the number of patients in an individual study; superscript is the reference. Some studies analyzed more than one group of patients.
[c]By Western blot method; all other protein studies used immunohistochemical methods.

TABLE 8. CORREL... WITH BREAST CAR... ...OUTCOME IN PATIENTS

P[a]	Type of c-erbB Activ	No. ...stasis	Statistical ...nalysis[a]	Reference
<0.05	DNA		M	87
<0.05	DNA		U	60
<0.05	DNA		U	65
<0.05	DNA		U	93
<0.05	mRNA		U	65
<0.05	Protein		M	101
<0.05	DNA		M	81
<0.05	DNA		U	17
<0.05	DNA		U	87
<0.05	DNA		M	79
<0.05	Protein		M	85
<0.05	Protein	44	U	101
0.05–0.15	DNA		U	111
0.05–0.15	Protein		M	92
0.05–0.15	Protein		U	86
>0.15	DNA		U	113
>0.15	DNA		M	4
>0.15	DNA		U	44
>0.15	mRNA		U	50
>0.15	Protein		M	66
>0.15	Protein		U	11
>0.15	Protein		U	39
>0.15	Protein		U	17
>0.15	DNA	181	M	81
>0.15	DNA	59	U	17
>0.15	DNA	73	U	87
>0.15	Protein	78	U	85
>0.15	Protein	92	U	17
>0.15	Protein	41	U	86
>0.15	Protein	41	U	40

[a]The endpoints of the ... c-erbB-2 activation and ... at 0.05 to 0.15, and is ...
[b]Shown as variable ... immunohistochemical ...
[c]M = multivariate stati... ...survival or both. Correlation between c... ...unt at <0.05, is of equivocal significance ...m blot; the other protein studies used ...

TABLE 6. PERCE
WITH PROGNOS
ITH METASTASIS COMPARED
/ATION

% of tumors with lymph node metastasis in each study	
64(DNA)[111]	64 (mRNA)[60] 61(DNA)[4]
	58 (DNA)[44] 57 (DNA)[113] 55(Protein)[39]
	48(Protein)[11] 46(Protein)[58]
42(Protein)[62]	
P<0.15	P>0.15

P for correlation of c-
Each study's percen
erbB-2 activation an
cancer patients, whe
are the types of c-erb

ompared with the correlation between c-
that considered, as one group, all breast
cripts are the references. In parentheses
able 3.

A second
together in mu
c-erbB-2 activa
infiltrating duc
erbB-2 activati
inflammatory b
erbB-2 activati
noma, but it is
A third pot
c-erbB-2 activa
metastasis. Tw
metastasis who
and absence o
recurrence.[23,67]
no association l
A fourth
correlates bett
overproduction

reast carcinoma are grouped
rrent literature suggests that
inoma, studies that combine
ite the prognostic effect of c-
most st dies do not analyze
ondition frequently shows c-
n the usual mammary carci-

dies that attempt to correlate
s of breast carcinoma without
patients without lymph node
nce (such as large tumor size
rexpre ion predicted early
situ, s small study found
B-2 activation.[40]
ding whether the prognosis
n or with mRNA or protein
n between c-erbB-2 activa-

tion and poor patient ou
and breast carcinoma pa
poorer survival.[70,81] Rece
tic power than overpro
overproduction without
studies have attempted
overproduction, and ma
tively less reliable meth
clonal antibodies.

Comparison of c-*erb*B
Breast Carcinoma

Other oncogenes that m
are reviewed elsewhere.
between the clinical rele

The c-*myc* gene is
tion generally has less pr
One study found a corre
myc, although other rep
however, could demons
more prognostic importa

The gene c-*erb*B-1
homologous with c-*erb*B
Overproduction of EGF
and may correlate with
*erb*B-2 and EGFR in the
poor prognostic factors.[35]
amplification of c-*erb*B-2
although at the molecul
protein.[51,52,61,68,100] Recer

The genes c-*erbA* a
tor, and they are locate
are frequently coamplifi
c-*erbA* expression in bre
tant role for this gene in
without *ear*-1 amplificat
similar to tumors with
c-*erb*B-2 amplification s
or *ear*-1.

Other genes also h
carcinomas. One study f
2 mRNA and increased
and Ki-*ras*.[105] Allelic de
breast carcinoma,[21] but i
studies have suggested
breast carcinoma and ac

A amplification (Table 5),
ion of c-*erb*B-2 may have
cation has more prognos-
significance of c-*erb*B-2
urther esearch.[17,52] Few
e with c-*erb*B-2 mRNA
overproduction use rela-
mical studies with poly-

ncogenes in

in human breast cancer
tricted to a comparison
her oncogenes.
omas, but c-*myc* activa-
B-2 activation.[21,34,77,87,93]
NAs of c-*erb*B-2 and c-
[96] Subsequent research,
mas in which c-*myc* has

tor receptor (EGFR) is
in breast carcinomas.[79]
ently gene amplification
have examined both c-
trong correlation with
no correlation between
of c-*erb*B-2 and EGFR,
phorylation of c-*erb*B-2
east carcinoma.[43,100]
hyro hormone recep-
osom 7. These genes
om The absence of
ence gainst an impor-
n of c-*erb*B-2 can occur
erea survival that is
catio Consequently,
amp cation of c-*erb*A

B-2 a vation in breast
ween creased c-*erb*B-
gro factor chain A,
a p er prognosis in
*erb*B- ctivation. Some
stag r recurrence of
coger [21,113]

ACTIVATION OF c

Y TISSUES

Incidence of c-er

Table 7 summarize

detected, usually w

mary Tissues

***c-erb*B-2 expression has been**

ethods using polyclonal anti-

TABLE 7. PRESENCE

NORMAL HUMAN TIS

A OR c-erbB-2 PROTEIN IN

Tissues With c-erbB-2 mRNA	Tis c-	ng A	Tissues Lacking c-erbB-2 Protein
Skin[24]	Epid Exter Eccrin		
	Fetal Fetal		Po fetal oral mucosa[62] Po fetal esophagus[62]
Stomach[24]	Stom Fetal		
Jejunum[24]	Smal		
Colon[25]	Colo		
Kidney[24]	Fetc		Gl rulus[62] Po fetal Bowman's capsule[62] Po fetal proximal tubule[62]
	Fetal Distal Fete Fet Fet		Po fetal collecting duct[62] Po fetal renal pelvis[62] Po fetal fetal ureter[62]
Liver[24]	Hep Par Par End of		Liver[62,65] Pa reatic islets[63]
Lung[24]	Fetal Fet Bro		Po fetal trachea[62] Po fetal bronchioles[62]
Fetal brain[24]	Fet		Po fetal alveoli[62,66] Po fetal brain[62] Po fetal ganglion cells[62]
Thyroid[1]			
Uterus[24]	Ov Blo		
Placenta[24]			Er helium[62]
			A rtical cells[62] P fetal thymus[62] F ists[62] S th muscle cells[62] Ca diac muscle cells[62]

[a]This protein study use

chemical n ods.

bodies. Only a few st ies ... and some of these do not demonstrate convinci ... in the published photographs. The interpret ... ver, are based, with the caveat that these find ... immunoprecipitation or Western or RNA blot ... been identified in normal epithelium of the gas. ... Discrepancies regarding c-*erb*B-2 protein in oth ... iss ... t in part, to differences in techniques.

The data on c-... B-2 ... non-mamm... neoplasms should be interpreted ... number ... tumors have been studied, usually ... ods using ... yclonal antibodies. Studies using ... because ... l culture can induce amplification ... ies, alth... this has not been documented for ...

Activation of c-... -2 ... 32 perce... of 203) of ovarian carcinomas in ... ht s ... stract[45] stu... that ovarian carcinomas contained ... mif ... protein tha... varian non-epithelial malignanc... ... t 12 per... of ovarian carcinomas had c-*erb*... ... ificatio...

Activation of c-... ... perc... of 198) of gastric adenocarcino... ... 33 pe... of 64) of ...

TABLE 8. c-*erb*B-2 AC...

Tumor Ty...	TUMORS: c-*erb*B-... mR N... C... pro...	c-*erb*B-2 Protein Over-production
Ovary—carcinoma, not ... specified	2...	23/73,[12] 38/72[91]
Ovary—serous (papillary)	—	—
Ovary—endometrioid ca...	...	—
Ovary—mucinous carcino...	...	—
Ovary—clear cell carcino...	...	—
Ovary—mixed epithelial		—
Ovary—endometrioid b...		—
Ovary—mucinous borde...		—
Ovary—serous cystadeno...		—
Ovary—mucinous cystad...	—	—
Ovary—sclerosing stro...	...	—
Ovary—fibrothecoma	—	—
Uterus—endometrial ad...	...	—

[a]Shown as number of case... ...pli... ...ber of... ...ed; reference is given as superscript. All pr... ...

intestinal or tul
subtypes (Table
281) of colorec
study detected
tion. One stud
protein in colon
using Boulin's fi
sive carcinoma
erbB-2 protein
nomas (46 of 63
some of these

TABLE 9. c-erbB-

		ESTINAL	ORS[a]
		erbB-2	c-erbB-2 Protein Over-production
Esophagus—squ			0/1[81]
Stomach—carcino			—
Stomach—adenoc		2/9,[	4/27,[39] 3/10[81]
Stomach—carcino			16/54[39]
Stomach—carcino			4/45[39]
Colorectum—carci		1/46	1/22,[39] 7/8[220]
Colon—villous ad			—
Colon—tubulovillo			—
Colon—tubular ad			19/19[220]
Colon—hyperplast			—
Intestine—leiomyo		—	0/1[81]
Hepatocellular carc			12/14,[36] 0/2[41]
Hepatoblastoma			—
Cholangiocarcinon			46/63[38]
Pancreas—adeno		—	2/80,[41a] 0/2[41]
Pancreas—acinar		—	0/1[41]
Pancreas—clear c		—	0/2[41]
Pancreas—large c		—	0/3[41]
Pancreas—signet		—	0/1[41]
Pancreas—chronic		—	0/14[41a]

[a]Shown as number of ... studied; reference is given as superscript. ... analyzed for c-erbB-2 mRNA.
[b]Tissues fixed in Bou
[c]Only cases with dis... duction.

...7) of di ... signet ring cell
...n dete ... 2 percent (6 of
...ional ... ohistochemical
...tissues ... in Bouin's solu-
...unical re ... ity for c-erbB-2
...e adjacen ... rmal epithelium,
...ures a ... ,ression to inva-
...histoch ... reactivity for c-
...14 cas ... cholangiocarci-
...st ... one study, but
... staining, which

TABLE 10. c-erbB-

Tumor Type	c-erbB-2 Protein Overproduction
Non-small cell carcir	1/84[53]
Epidermoid carcinon	3/5[56]
Adenocarcinoma	7/12[56]
Large cell carcinom	—
Small cell carcinom	26,[53] 0/3[56]
Carcinoid tumor	3[56]

[a]Shown as number of c … given as superscript. Al … 2 mRNA.

does not indicate … atic carcinomas … chemical reactivi … atic adenocarcinc…

Tables 10 th … neoplasms. The … Activation of c-e… non-small cell c… found c-*erb*B-2 p… noma had c-*erb*b… tion of c-*erb*B-2 … urinary bladder, … cell carcinoma o…

…s.[95] Al … some pancre- … toplas … immunohisto- … the r … ce of pancre- …g.[4] …B- … tion in other … mos… …ese tumors. …4 or … of pulmonary … one ad… …onal report[99] … of 17). …ul cell carci- … r stu… Overproduc- …al c… …noma of the …ell …ma and basal …In … is not clear

TABLE 11. c-erbB-

Tumor Ty…	RO…	…ONS[a] c-erbB-2 mRNA …	c-erbB-2 Protein Over-production
Hematologic malign…	—		—
Malignant lymphom…	1		0/15[51]
Acute leukemia	—		—
Acute lymphoblastic …	—		—
Acute myeloblastic …	—		—
Chronic leukemia	—		—
Chronic lymphocytic …	—		—
Chronic myelogenous …	—		—
Myeloproliferative d…	—		—

[a]Shown as number of … given as superscript. Al… …ed; reference is …

TABLE 12. c-erb ... OFT ... AND BONE*

	...-2 D... ...lcatic...	
...ur...	...0/8...	
...d...		
	...2/2...	
...or		
...ir		
...n		

*Shown as number of ... umber o... studied; reference is given as superscript. ... 2 protei...

whether the p... f nor... kin.[56] Thyroid carcinomas and ... rbB-2 mRNA. One abstract d... ...plif... in one of ten salivary gland p...

Correlation of ...ute...

Very few stud... rbB- ...vation in non-mammary tumo... d th... ...bB-2 amplification or overexpr... ...s with... ...eased survival, especially wh... not report the stage, histologic... ...asms. Another study of stages ... ...ation between decreased surviv... on, ... between survival and histolo... ...erb... ...rotein overproduction in 10 ... orrel... with decreased disease-free int... ...nd ... immunohisto-

TABLE 13. c-e ... TRACT*

Tumor T...	...	c-erbB-2 Protein Over-production
Kidney—renal ...		—
Wilms' tumor		—
Prostate—adeno...	—	0/23[?]
Urinary bladder...		1/48[?]

*Shown as numbe... ...studied; reference is given as superscr...

TABLE 14. c-erbB-2 A T

Tumor T[illegible]	-2 mRNA oduct	c-erbB-2 Protein Overproduction
Skin—malignant m[illegible]	—	0/10[a]
Skin, head and nec[illegible] cell carcinoma	—	—
Site not stated—sc[illegible] carcinoma	—	—
Salivary gland—adenoc[illegible]	—	—
Parotid gland—adenoid c[illegible] carcinoma	—	0/1[a]
Thyroid—anaplasti[illegible]	—	—
Thyroid—papillary [illegible]	evels)/5	—
Thyroid—adenocar[illegible]	—	—
Thyroid—adenoma	vels)/2	—
Neuroblastoma	—	—
Meningioma	—	—

[a]Shown as number of c[illegible] given as superscript. A[illegible]

of cases	id; references

chemical reactiv[illegible] higher [illegible]es of pros-
tatic adenocarci[illegible] varian[illegible] nomas and
other neoplasms [illegible]

SUMMARY

Activation of th[illegible] DNA and by ove[illegible] mately 20 perce[illegible] which correlates [illegible] axillary lymph n[illegible] tion with other p[illegible] conclusions. The [illegible] other neoplasms [illegible]

[illegible]plificati[illegible] of c-erbB-2
[illegible]B-2 pr[illegible]. Approxi-
[illegible] c-er[illegible] activation,
[illegible]its wi[illegible]tastasis to
[illegible]late [illegible]B-2 activa-
[illegible]repu[illegible] conflicting
[illegible]-erbB-[illegible]ivation in
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